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RESEARCH ARTICLE

Growth stage of *Phalaris minor* Retz. and wheat determines weed control and crop tolerance of four post-emergence herbicides

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Abstract

Phalaris minor Retz. has evolved multiple herbicide resistance in wheat growing areas in northwestern India. An understanding of the effect of growth stage on herbicide tolerance of wheat and control of *P. minor* will help in selecting the most appropriate herbicide for different situations. The weed control and crop safety of four commonly used wheat herbicides (sulfosulfuron, pinoxaden, fenoxaprop plus metribuzin and mesosulfuron plus iodosulfuron), each applied at four different wheat growth stages was investigated in field studies for two years. *P. minor* plants were at 1, 2-3, 3-4 and 7-8 leaf stages when the herbicides were applied at Zadok 12-Z12, Z13, Z21 and Z23 stages of wheat, respectively. Sulfosulfuron application at Z12 and Z13 wheat stages (before first irrigation), provided >80% control of *P. minor* and produced wheat grain yield (4.5-4.7 t/ha) similar to the weed-free check (4.9 t/ha) in both years. Pinoxaden, fenoxaprop plus metribuzin and mesosulfuron plus iodosulfuron application at Z12 and Z13 wheat stages recorded significantly lower wheat grain yield (3.62-3.95 t/ha) due to poor weed control, crop toxicity or both. All the four herbicides were equally effective on *P. minor* when applied at Z21 wheat stage. At Z23 wheat stage, pinoxaden gave >90% control of *P. minor* and the highest wheat grain yield (4.82 t/ha). The results are expected to allow changes in the current recommendation of the timing of post-emergence herbicides for the management of *P. minor* in wheat.

Additional key words: crop toxicity; crop stages; littleseed canarygrass; selectivity; *Triticum aestivum*.

Abbreviations used: ALS (acetolactate synthase); DAS (days after sowing); DAT (days after treatment); Z (Zadoks).

Authors' contributions: Conceived and designed the experiment; data analysis and interpretation; publication drafting: RR, MSB, GSG. Performed the experiments: RR, MSB.

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Introduction

Littleseed canarygrass (*Phalaris minor* Retz.) is one of the most troublesome annual weeds of wheat (*Triticum aestivum* L.) in northwestern India since many years ago (Bhullar *et al.*, 2016). Globally, it has been reported in more than 60 countries, covering all the continents (Singh *et al.*, 1999). The weed is highly competitive with wheat and can reduce yield by up to 95% (Chhokar *et al.,* 2006). It germinates in different flushes from sowing, to well after first and second irrigations. According to the current recommendation, *P. minor* in wheat in northwestern India, can be controlled with pre-emergence herbicides applied within 2 days after sowing (DAS) or with post-emergence herbicides at 30 DAS; post-emergence herbicides are currently most popular (>95%) with the farmers in the region. Rice-wheat is the dominant cropping system in northwestern India, and *P. minor* often germinates in moist seedbed at the same time as wheat. However in some seasons, rainfall during wheat sowing can stimulate early germination of weeds. Under these situations, *P. minor* can reach an ideal stage for herbicide application (2-3 leaf) even though wheat crop is not at the labelled stage (Zadoks 21-Z21). At present, the consequences of spraying wheat earlier than the currently recommended stage (Z21) are unclear.

Correct timing of herbicide application plays an important role in achieving effective weed control without causing crop injury. Crop tolerance to herbicides varies with herbicide choice, application dose, application timing, and environmental conditions (Lemerle *et al.,* 1986). Growth stage of wheat and environmental conditions at the time of herbicide application also affect herbicide uptake, translocation and plant's ability to metabolize herbicides thus affecting the extent of herbicide injury and weed control (Tottman, 1980). Sulfosulfuron, pinoxaden, pre-mixes of fenoxaprop plus metribuzin and mesosulfuron plus iodosulfuron are commonly used for *P. minor* control in wheat in the region. Sulfosulfuron is a sulfonylurea herbicide that inhibits the activity of acetolactate synthase (ALS), an important enzyme necessary for the biosynthesis of branched chain amino acids. It has been widely used locally for the control of *P. minor*. There have already been some reports of resistance to sulfosulfuron in *P. minor* in some areas of Punjab (Bhullar *et al.*, 2014) and Haryana (Dhawan *et al.*, 2010) states in northwestern India. The pre-mix of mesosulfuron-methyl and iodosulfuron-methyl-sodium (ALS inhibitor) is also widely used in wheat. GR_{50} (dose required for 50% growth reduction) values of mesosulfuron plus iodosulfuron have been reported to increase with time (AICRPWM, 2013). More recently pinoxaden, a selective grass herbicide belonging to the phenlypyrazolin chemistry, has been recommended for post-emergence control of *P. minor*. Dhawan *et al.* (2010) and Kaur *et al.* (2016) reported development of cross resistance in *P. minor* to pinoxaden in Punjab and Haryana states. Metribuzin is primarily a soil-residual herbicide, which can be applied post-emergence to wheat for the control of annual grasses and dicot weeds (Schroeder *et al.,* 1986; Shaw & Wesley, 1991). Although metribuzin can control *P. minor* effectively, careful management, including timely application is required to achieve good crop tolerance and weed control (Runyan *et al.,* 1982; Schroeder *et al.,* 1986). The pre-mix of fenoxaprop-p-ethyl plus metribuzin can provide effective weed control but it can also cause some phytotoxic effects on the tillering and grain yield of wheat (Singh *et al*., 2005). Even though there have been some reports of resistance to sulfosulfuron, pinoxaden and pre-mix of mesosulfuron plus iodosulfuron in *P. minor* in northwestern India, these herbicides are still extensively used in the region. An understanding of the effect of growth stage on herbicide tolerance of wheat and control of *P. minor* will help in developing recommendations for selecting the most appropriate herbicide for different situations. The objective of this study was to evaluate the effect of timing of different post-emergence herbicides on crop tolerance and their efficacy on *P. minor* control.

Material and methods

Description of study site

Field experiments were conducted under irrigated conditions at the research farm of Punjab Agricultural University, Ludhiana, India during winter season of 2013-14 and 2014-15. The experimental soil was sandy loam with organic carbon (0.39%), pH (8), EC (0.13 mmhos/cm) and was low in available nitrogen (214 kg N/ha), available phosphorous (9 kg P/ha) and high in available potassium (337 kg K/ha). The experimental field had been under irrigated rice-wheat cropping system and was planted with dry-seeded rice in the summer seasons. The weekly weather data (maximum and minimum temperature and rainfall) during the two growing seasons are represented in Fig. 1. The wheat crop received 172 mm rain in 2013-14 and 221 mm in 2104-15.

Experimental design and crop management

A heavy pre-sowing irrigation (10 cm) was applied to ensure adequate moisture in the soil at the time of planting. Seedbed preparation consisted of ploughing with a disc harrow followed by two passes with a field cultivator followed by planking. Seeds of *P. minor* were broadcast uniformly in the field before the last cultivation. Wheat (cv. HD-2967) was seeded on November 19, 2013 and November 12, 2014 using seed rate of 100 kg/ha, in 22.5 cm spaced rows. The crop was fertilized by using Urea as the source of nitrogen at 125 kg N/ha in two splits; half as basal and half with the first irrigation. Phosphorous was applied at 62.5 kg P_2O_5/ha through Single Super Phosphate and potassium at 30 kg K_2O/ha through Muriate of

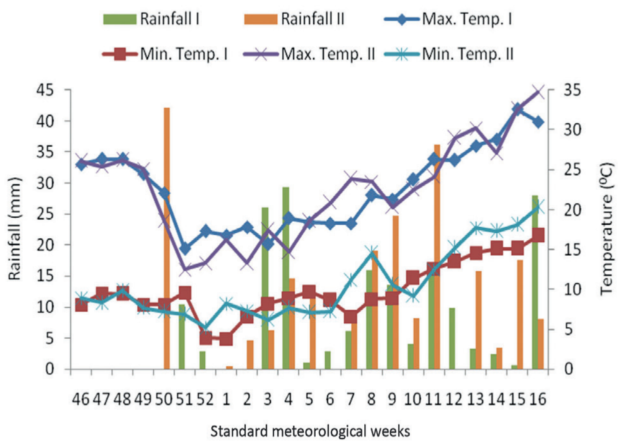

Figure 1. Average weekly weather data for two cropping seasons, 2013-14 (I) and 2014-15 (II), at Punjab Agricultural University Research Farm near Ludhiana, India.

Table 1. Effect of different weed control treatments on density and biomass of *P. minor* during 2013-14 (I) and 2014-15 (II) growing seasons at Punjab Agricultural University Research Farm near Ludhiana, India.

Treatment	Wheat stage[1]	*P. minor* density (plants/m^2)						*P. minor* biomass (g/m^2)					
		30 DAS		45 DAS		135 DAS		45 DAS		90 DAS		135 DAS	
		I	II	I	II	I	II	I	II	I	II	I	II
Sulfosulfuron 25 g/ha	Z12	19	12	13	9	17	14	0.8	0.7	5.9	5.4	28.3	20.3
Pinoxaden 50 g/ha	Z12	42	38	82	75	110	96	5.2	4.7	44.2	39.1	147.2	134.5
Fenoxaprop+Metribuzin 275 g/ha	Z12	12	8	4	3	8	9	0.1	0.1	3.6	3.0	7.0	5.7
Mesosulfuron+Iodosulfuron 14.4 g/ha	Z12	44	33	52	44	71	58	2.9	2.7	27.9	22.9	93.2	78.7
Sulfosulfuron 25 g/ha	Z13	18	9	8	7	14	12	0.6	0.6	4.8	4.6	19.2	17.8
Pinoxaden 50 g/ha	Z13	27	37	44	43	63	56	2.8	2.6	26.5	22.8	84.3	75.3
Fenoxaprop+Metribuzin 275 g/ha	Z13	8	6	3	3	6	5	0.1	0.1	3.5	2.8	3.8	3.5
Mesosulfuron+Iodosulfuron 14.4 g/ha	Z13	47	37	39	37	57	42	2.8	2.5	24.6	22.0	74.7	65.4
Sulfosulfuron 25 g/ha	Z21	49	43	7	6	9	4	0.5	0.5	3.7	3.0	13.4	8.6
Pinoxaden 50 g/ha	Z21	68	45	5	5	6	8	0.2	0.1	3.4	2.9	6.4	5.3
Fenoxaprop+Metribuzin 275 g/ha	Z21	57	63	3	2	6	3	0.1	0.1	3.2	2.6	3.5	3.3
Mesosulfuron+Iodosulfuron 14.4 g/ha	Z21	64	50	10	7	14	11	0.5	0.5	4.7	4.5	20.9	14.1
Sulfosulfuron 25 g/ha	Z23	54	46	132	130	16	14	5.1	4.8	7.1	6.9	26.4	20.1
Pinoxaden 50 g/ha	Z23	65	47	140	127	12	7	7.4	7.3	7.6	7.4	13.9	9.2
Fenoxaprop+Metribuzin 275 g/ha	Z23	53	47	136	126	33	24	7.3	6.8	21.1	15.6	37.1	29.5
Mesosulfuron+Iodosulfuron 14.4 g/ha	Z23	58	48	135	133	25	20	6.9	6.9	15.5	7.6	31.4	24.3
Weedy check	-	61	51	127	124	258	250	7.4	6.9	68.5	60.0	195.6	192.0
LSD ($p \leq 0.05$)	-	15	13	11	8	9	7	0.5	0.4	3.7	4.5	10.3	14.2

[1] Wheat stage at time of spray. Z: Refers to the Zadok's stage of wheat. DAS: days after sowing. Data were square-root transformed before analysis; however, back-transformed actual mean values are presented based on the interpretation from the transformed data.

Potash as basal dose. Each year, the experiment was conducted in a randomized complete block design with three replications. Sixteen treatments consisted of four herbicides viz. sulfosulfuron at 25 g/ha, pinoxaden at 50 g/ha, pre-mixes of fenoxaprop plus metribuzin at 275 g/ha and mesosulfuron plus iodosulfuron at 14.4 g/ha, each applied at Z12 (14 DAS), Z13 (21 DAS), Z21 (30 DAS) and Z23 (45 DAS) (Zadoks *et al.,* 1974) (Table 1). A weed-free check and a weedy check was also included for comparison with the herbicide treatments. The gross plot area was 3.25 m × 7 m and net plot was 2.02 m × 5.2 m. The herbicide doses used in the study are currently recommended for the control of *P. minor* in wheat in the region at 30 DAS. At Z12, Z13, Z21 and Z23 stages of wheat, *P. minor* plants were at 1, 2-3, 3-4 and 7-8 leaf stages, respectively. All herbicides were applied using knapsack sprayer fitted with a flat fan nozzle which was calibrated to deliver 375 L/ha of spray solution. The crop was supplied with four post-sowing irrigations (each 7.5 cm) at 23, 58, 90 and 121 DAS during 2013-14 and at 23, 63, 88 and 128 DAS during 2014-15. The irrigation was applied when the soil moisture had declined to one-third of field capacity. All management practices other than the weed control treatments were consistent with the recommended package of practices for the region. In the weed free treatment, weeds were removed by hand weeding throughout the season. *P. minor* was the major weed in the field; a few broad leaf weed plants present in the field were removed by hand pulling.

Data collection

Phalaris minor plant density was assessed, by using a 0.45 m^2 quadrant placed at two representative locations, in each plot at 30, 45 and 135 (tiller density) DAS. *P. minor* plants were cut and dried in an oven at 60^{o}C for 72 h and the above ground biomass recorded at 45, 90 and 135 DAS. The visible crop injury was assessed at 7, 14 and 21 days after herbicide treatment (DAT) and rated on 0-100% scale (0=no toxicity; 100=complete killing). To further quantify wheat response to herbicides, plant height was measured from the ground to the tip of the uppermost leaf from six randomly selected plants in each plot at 45 DAS, and spikes were counted in 1-m^2 at two locations in each plot at 90 DAS and at crop harvest (productive spikes). Grain weight/spike was determined from six representative spikes from each plot; these spikes were then used to obtain number of grains/spike. The crop was manually harvested at maturity on 20 April, 2014 and 22 April, 2015.

Statistical analyses

Data were analyzed using the GLM procedure in SAS 9.3 to evaluate the differences between treatments (SAS, 2001). Normality, homogeneity of variance, and interactions of treatments and years were tested. In this study, year by treatment interactions were significant; therefore, data were presented separately for each year. Weed density and biomass data were square root transformed and herbicide injury data were arc-sine transformed before performing ANOVA because of high variance. However, non-transformed means are presented with mean separation based on transformed values. Weed free check was not included in the analysis of crop injury, however, all values were compared independently to zero to evaluate treatment differences with the weed free check. Where the ANOVA indicated that treatment effects were significant, means were separated at $p<0.05$ and adjusted with Fisher's Protected Least Significant Difference (LSD) test.

Results

Control of *P. minor*

The *P. minor* density in the weedy check increased from 51-61 plants at 30 DAS to 124-127 plants/m^2 at 45 DAS and then stabilized (Table 1). This indicated that 45% of *P. minor* germinated before 30 DAS and 55% between 30-45 DAS. Application of sulfosulfuron and fenoxaprop plus metribuzin at Z12 and Z13 wheat stages had significantly lower density of *P. minor* at 135 DAS (12-17 and 5-9 plants/m^2, respectively), compared to pinoxaden (56-110 plants/m^2) and mesosulfuron plus iodosulfuron (42-71 plants/m^2). When applied at Z21, all herbicide treatments significantly reduced *P. minor* density (2-14 plants/m^2) compared to the unsprayed check (250-258 plants/m^2). When applied at Z23 wheat stage, pinoxaden recorded lower weed density (7-12 plants/m^2) than the other three herbicides ($p<0.05$).

Phalaris minor biomass in the weedy check recorded the highest increase (> 9 times from 7 to 64 g/m^2) between 45- 90 DAS; it increased further by 3-fold (64 to 194 g/m^2) from 90-135 DAS (Table 1). The application of different herbicides reduced *P. minor* biomass at 135 DAS by 25-98% as compared to the weedy check (192-196 g/m^2). When applied at Z12 and Z13 wheat stages, fenoxaprop plus metribuzin had the lowest *P. minor* biomass (3-7 g/m^2) followed by sulfosulfuron (18-28 g/m^2), as compared to significantly higher biomass under pinoxaden (75-147 g/m^2) and mesosulfuron plus iodosulfuron (65-93 g/m^2) ($p<0.05$). When applied at Z21 stage, all the herbicides recorded similar biomass of *P. minor*, which was significantly lower than the weedy check. Pinoxaden applied at Z23 stage had a significantly lower *P. minor* biomass (9-14 g/m^2) than the other herbicides (20-37 g/m^2).

Wheat growth and development

Application of fenoxaprop plus metribuzin at Z12 and Z13 wheat stages caused toxicity to wheat plants in both years (Fig. 2). The toxicity symptoms, wilting of foliage followed by whitening/bleaching of the leaf tissue, appeared after the first irrigation and were more severe when the herbicide was applied at Z13 than at Z12 stage. The treatment at Z13 caused 36-38% toxicity at 7 DAT, which reduced to 5-6% at 14 DAT and to 0-2% at 21 DAT. Application of fenoxaprop plus metribuzin at Z12 stage showed 0% toxicity at 7 DAT, 26-28% at 14 DAT which reduced to 2% at 21 DAT. The growth of the surviving crop plants in fenoxaprop plus metribuzin treatment was stunted, and herbicide toxicity reduced wheat plant density by 23-25% at 90 DAS when the spray was done at Z12 and Z13 stages. Application of mesosulfuron plus iodosulfuron at Z12 and Z13 stages suppressed crop growth, caused interveinal chlorosis of leaves and minor necrosis of leaf tips of wheat plants. The toxicity symptoms were more severe when this herbicide treatment was applied at Z13 (19-22%) compared to at Z12 (12%). Pre-mixes of fenoxaprop plus metribuzin and mesosulfuron plus iodosulfuron were safe to wheat

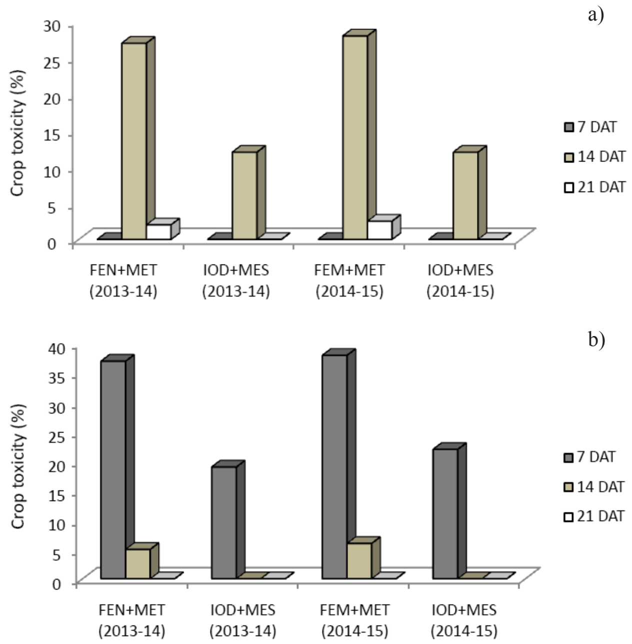

Figure 2. Toxicity of pre-mixes of fenoxaprop (FEN) plus metribuzin (MET), and of mesosulfuron (MES) plus iodosulfuron (IOD) on wheat plants when applied at Zadok 12-Z12 (a) and Z13 (b) wheat stages; pinoxaden and sulfosulfuron were safe in both years.

Table 2. Effect of different weed control treatments on growth, yield attributes and grain yield of wheat, during 2013-14 (I) and 2014-15 (II) growing seasons at Punjab Agricultural University Research Farm near Ludhiana, India.

Treatment	Wheat stage[1]	Plant height (cm) (45 DAS)		Plant density/m² (90 DAS)		Spikes at harvest/m²		Grains/ spike		Grain weight/ spike (g)		Grain yield (t/ha)	
		I	II	I	II	I	II	I	II	I	II	I	II
Sulfosulfuron 25 g/ha	Z12	17.9	20.7	327	335	280	283	48	50	2.0	2.5	4.45	4.57
Pinoxaden 50 g/ha	Z12	17.7	20.1	246	263	189	190	35	36	1.0	1.5	2.78	2.85
Fenoxaprop+Metribuzin 275 g/ha	Z12	14.2	15.7	287	295	219	222	48	50	2.0	1.9	3.66	3.85
Mesosulfuron+Iodosulfuron 14.4 g/ha	Z12	14.9	18.4	316	325	250	254	37	39	2.0	1.8	3.70	3.95
Sulfosulfuron 25 g/ha	Z13	18.1	20.8	334	347	290	292	52	52	2.5	2.7	4.69	4.78
Pinoxaden 50 g/ha	Z13	17.8	20.7	303	315	220	229	39	39	1.6	1.8	3.69	3.90
Fenoxaprop+Metribuzin 275 g/ha	Z13	13.2	15.4	284	286	205	213	47	49	1.7	1.9	3.62	3.71
Mesosulfuron+Iodosulfuron 14.4 g/ha	Z13	14.7	17.6	310	318	248	252	36	38	1.5	1.8	3.68	3.89
Sulfosulfuron 25 g/ha	Z21	19.0	21.0	360	372	303	304	54	55	2.6	2.8	4.82	4.87
Pinoxaden 50 g/ha	Z21	19.5	21.1	363	378	304	305	54	56	2.6	2.9	4.87	4.93
Fenoxaprop+Metribuzin 275 g/ha	Z21	19.5	21.6	369	382	304	306	55	57	2.7	2.9	4.91	4.95
Mesosulfuron+Iodosulfuron 14.4 g/ha	Z21	18.7	20.9	350	366	296	297	50	54	2.5	2.7	4.73	4.81
Sulfosulfuron 25 g/ha	Z23	17.3	20.0	347	356	289	292	50	52	2.4	2.6	4.67	4.66
Pinoxaden 50 g/ha	Z23	16.9	20.1	357	368	296	298	52	55	2.5	2.7	4.79	4.82
Fenoxaprop+Metribuzin 275 g/ha	Z23	17.7	20.4	322	329	256	259	40	41	2.1	2.3	4.18	4.35
Mesosulfuron+Iodosulfuron 14.4 g/ha	Z23	17.6	20.2	343	351	285	289	48	52	2.4	2.6	4.63	4.75
Weed free check (hand weedings)	-	19.7	22.1	373	384	306	307	55	58	2.8	3.0	4.95	4.98
Weedy check	-	17.0	20.7	240	250	182	184	32	33	1.4	1.0	2.44	2.50
LSD ($p \leq 0.05$)	-	2.9	3.5	37	38	31	34	8	9	0.5	0.5	0.69	0.64

[1] Wheat stage at time of spray. Z: Refers to the Zadok's stage of wheat. DAS: days after sowing.

when applied at Z21 and Z23 stages. Sulfosulfuron and pinoxaden did not cause any phytotoxicity to wheat plants when applied at any of the stages of wheat.

Wheat grain yield and yield attributes

The application of herbicides at different growth stages of wheat had a significant effect on wheat grain yield and yield attributes (Table 2). When herbicide applications were made at Z12 and Z13 stages, only sulfosulfuron provided yield levels (4.5-4.7 t/ha) similar to the weed-free check (4.9 t/ha) ($p>0.05$). Mesosulfuron plus iodosulfuron at Z12 and Z13 stages reduced wheat grain yield by 21 and 25%, respectively, as compared to the weed-free check. In case of fenoxaprop plus metribuzin, the yield reduction was 22-26% when application was made at Z12 and 25-27% at Z13. Early application of pinoxaden caused the highest grain yield reductions in wheat (42-44% at Z12 and 22-34% at Z13). All herbicides in this study recorded wheat grain yield at par to the weed-free check when applied at Z21 stage. Pinoxaden produced the highest grain yield when herbicides were applied at Z23 stage.

A negative relationship was observed between wheat grain yield and *P. minor* biomass in the linear regression model (Fig. 3; Table 3). The regression model explained about 98% of the variation in grain yield due to weed biomass and provided a good fit between the grain yield and the weed biomass. Pre-mix of fenoxaprop plus metribuzin applied at Z12 and Z13 wheat stages was excluded from the regression model, as the grain yields under these treatments were influenced by crop toxicity

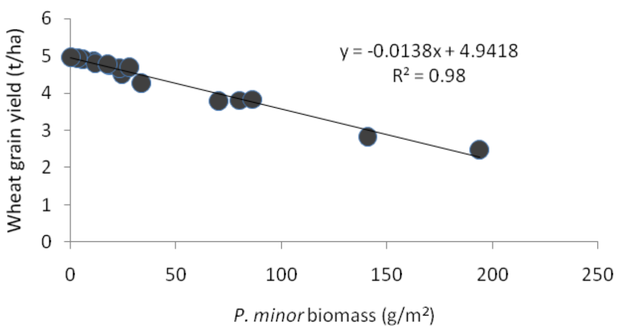

Figure 3. Relationship between the wheat grain yield and *Phalaris minor* biomass. The line represents a linear model fitted to the average of the data for both years.

and not just weed control. In all the other weed control treatments, grain yield reduction was mainly influenced by *P. minor* biomass.

Discussion

Sulfosulfuron and fenoxaprop plus metribuzin provided effective control of *P. minor* when applied at Z12 and Z13 wheat stages, all four herbicides were equally effective when applied at Z21, and only pinoxaden worked effectively at Z23 wheat stage. Sulfosulfuron is absorbed both through root and foliage (Senseman, 2007) and has a broad window of application in wheat and longer residual effects (Shaner, 2014). *P. minor* plants were at 1-4 leaf stages, when herbicides were applied between Z12-21 stages of wheat, and would have been killed through both root and foliage uptake. The efficacy of sulfosulfuron against 2-3 leaf stages of *P. minor* has been reported earlier (Chhokar & Malik, 2002; Chhokar *et al.,* 2008). In contrast, Singh *et al*. (2003) recorded higher control of *P. minor* with sulfosulfuron used at Z13 compared to at Z21 wheat stage at Pantnagar, India. The results of this study suggest that even in situations where weeds germinate early after first irrigation, the current recommendation for sulfosulfuron at Z21 stage still provides effective weed control and crop yield response.

Fenoxaprop and metribuzin have contrasting modes of entry into plants; metribuzin enters the plant mainly from the soil via roots and fenoxaprop enters entirely through the foliage; their joint action may be the reason for enhanced efficacy against *P. minor,* particularly when it was applied at Z12-13 wheat stages. In an earlier study, Chhokar *et al.* (2008) reported effective control of *P. minor* with metribuzin applied at Z23 stage of wheat, but in our study metribuzin plus fenoxaprop had a lower efficacy than its earlier timing, which may be attributed to the large size of *P. minor* plants (7-8 leaf stage). The lower efficacy of the mesosulfuron plus iodosulfuron on small plants of *P. minor* when applied at Z12-13 wheat stages, may be due to low leaf area of weeds as these herbicides are primarily absorbed through foliage (Yuan *et al.,* 2013) and known for more consistent results from later applications (Tickes, 2000; Crooks *et al.,* 2004). In an earlier study, mesosulfuron provided better control of *Lolium perenne* at 4-5 leaf than at 2-3 leaf stage (Grey *et al.,* 2012).This was also supported by our results where the best control of *P. minor* (>85%) with meosulfuron plus iodosulfuron was recorded at more than 3-leaf stage. Pinoxaden is also absorbed through the foliage only (Hofer *et al.,* 2006), hence its lower efficacy when applied at Z12 and Z13 wheat stages could be related to lower leaf area of *P. minor*, and its failure to prevent germination of *P. minor* seeds after its application. Consequently, pinoxaden worked most effectively once all target grasses had fully emerged. Therefore, the best weed control with this herbicide was observed when it was applied at Z21-23 wheat stages. Kieloch *et al.* (2006) indicated that pinoxaden can provide high levels of weed control even at late weed growth stage which was also supported by this study.

Wheat plants showed excellent tolerance to sulfosulfuron and pinoxaden at all growth stages. In the case of pinoxaden, the safener (cloquintocet-mexyl) present in the formulation provides very good tolerance in wheat (Hofer *et al.,* 2006). The pre-mixes of fenoxaprop plus metribuzin and mesosulfuron plus iodosulfuron were toxic to wheat plants at Z12 and Z13 stages and significantly reduced spike density compared to weed free check ($p<0.05$). These results are supported by the chlorophyll fluorescence (Fv/Fm) data, which showed reduced chlorophyll fluorescence in wheat plants compared to unsprayed plants indicating that the treated plants were under stress (data not shown). The higher crop toxicity by these herbicide mixtures when used at Z13 than at Z12 stage could be related to the short time interval between herbicide application and irrigation which increased herbicide uptake. The herbicide application at Z13 stage was followed by irrigation after 24 hours, and 10 and 42 mm rainfall was received one week after the spray during 2013 and 2014, respectively (Fig. 1). The close association between metribuzin toxicity in crops and soil moisture

Table 3. Correlation (r) among growth parameters associated with crop-weed competition.

Parameters [1]	Weed DM 45 DAS		Weed DM 90 DAS		Weed DM 135 DAS		Spikes/m^2		Grains/spike	
	I	II	I	II	I	II	I	II	I	II
Weed DM 90DAS	0.62	0.55								
Weed DM 135 DAS	0.53	0.49	0.98	0.99						
Spikes/m^2	-0.28	-0.26	-0.73	-0.75	-0.72	-0.75				
Grains/spike	-0.52	-0.45	-0.89	-0.88	-0.88	-0.87	0.81	0.82		
GY	-0.34	-0.33	-0.85	-0.87	-0.85	-0.87	0.97	0.97	0.89	0.87

[1] DM = weed biomass; GY = wheat grain yield. Values of $r>0.46$ and $r>0.57$ are significant at $p\leq0.05$ and at $p\leq 0.01$, respectively.

has been reported earlier (Blackshaw *et al.,* 1994; Kleemann & Gill, 2008). Crooks *et al.* (2003) reported toxicity to wheat plants 2-4 weeks after the treatment with mesosulfuron plus iodosulfuron and the toxicity reduced significantly when herbicide application was delayed to the tillering stage of wheat (Grey *et al.,* 2012). These studies suggest that tolerance of wheat to this herbicide increases close to the tillering stage. In our case, the crop also showed excellent tolerance to both pre-mixes when applications were made at Z21-23 stages which represent the tillering stage of wheat, and may be attributed to the enhanced capacity of wheat plants to metabolise the herbicides at these stages (Das, 2014). The differential sensitivity of wheat to metribuzin applied at its different growth stages (Shaw & Wesley, 1991; Grey & Bridges, 2003) and different doses (Balyan, 1999) has been demonstrated earlier.

The higher grain yield with sulfosulfuron, when applied at Z12 and Z13 wheat stages than later in the season, could be attributed to its excellent crop safety and lower biomass of *P. minor*. The selectivity of pre- and post- emergence sulfosulfuron in wheat was reported by Levy (2008). The results of our study indicated that sulfosulfuron can be adopted for the control of *P. minor* at these stages (Z12-13). At those early stages of wheat, fenoxaprop plus metribuzin showed toxicity, pinoxaden had poor weed control and mesosulfuron plus iodosulfuron showed both (toxicity and poor weed control) (Table 1; Fig. 2). The crop toxicity and/or poor weed control reduced wheat plant density, spike density and grains per spike, which lowered the grain yield by 21-44% compared to the weed free check ($p<0.05$) (Table 2). These results are supported by the strong positive correlation among spike density and grains per spike with grain yield ($p<0.01$) (Table 3). The weed biomass at 90 and 135 DAS are strongly correlated and has strong negative effects on spikes/m^2 and grain number per spike as well as grain yield ($p<0.01$). Hence treatments that provided poor weed control allowed large weed biomass, which resulted in lower grain yield. Further, grains/spike and grain number/m^2 are strongly related to grain yield, which also explain the differential response among different weed control treatments. The yield reduction with both the pre-mixes was greater when applied at Z13 than at Z12, which could be related to greater herbicide absorption associated with higher leaf area of the crop.

All four herbicides applied at the Z21 crop stage provided effective control of *P. minor* and were safe to wheat, and produced wheat grain yield similar to the weed-free check ($p>0.05$). When herbicide treatments were applied at Z21 stage, *P. minor* and wheat plants were within the recommended 3-4 leaf stage, which could be the reason for high herbicide efficacy and selectivity. Effective control of *P. minor* at Z21 wheat stage with these herbicides was also reported earlier (Chhokar & Malik, 2002; Singh *et al.,* 2005; Chhokar *et al.,* 2008). Pinoxaden provided excellent control (>90%), even with large plants of *P. minor* (7-8 leaf), which was the reason why it provided wheat grain yield similar to weed free check when applied at Z23 wheat stage. Weed biomass for the other three herbicides was greater than with pinoxaden, which could be associated with the differences in wheat grain yield. Singh *et al.* (2011) found higher *P. minor* control with pinoxaden compared to the pre-mix of fenoxaprop plus metribuzin when the herbicide application was made at wheat stages beyond Z23 stage. The negative linear relationship between wheat grain yield and *P. minor* biomass explained about 98% of the variation in grain yield (Fig. 3; Table 3). The application of different herbicides reduced *P. minor* biomass by 25-98% as compared to the weedy check (192-195 g/m^2). This level of competition from *P. minor* in the weedy check plots reduced crop density, spike density (35-40%) and wheat grain yield by 50% as compared to the weed free check.

As conclusions, the post-emergence application of 25 g/ha sulfosulfuron at Z12 and Z13 stages of wheat provided effective control of *P. minor* and was safe to wheat. Pre-mixes of fenoxaprop plus metribuzin and mesosulfuron plus iodosulfuron were toxic to wheat plants when applied at these stages. Pinoxaden application at 50 g/ha provided effective control of large *P. minor* (7-8 leaf). This study provides background information required to make changes to the current recommendation of timing of post-emergence herbicides in wheat in northwestern India.

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References

AICRPWM, 2013. Annual Technical Report of All India Coordinated Research Project on Weed Management. Punjab Agric. Univ., Dept. Agron., Ludhiana, India, 100 pp.

Balyan RS, 1999. Efficacy of sulfosulfuron, tralkoxydim and metribuzin applied alone and tank-mix on weeds in wheat. Indian J Weed Sci 31: 80-83.

Bhullar MS, Punia SS, Tomar SS, Singh VP, Sharma JD, 2014. Littleseed canarygrass resistance to clodinafop in Punjab: Farmers' perspective. Indian J Weed Sci 46: 237-240.

Bhullar MS, Pandey M, Kumar S, Gill G, 2016. Weed management in conservation agriculture in India. Indian J Weed Sci 48: 1-12. https://doi.org/10.5958/0974-8164.2016.00001.0

Blackshaw RE, Moyer JR, Kozub GC, 1994. Efficacy of downy brome herbicides as influenced by soil properties. Can J Plant Sci 74: 177-183. https://doi.org/10.4141/cjps94-037

Chhokar RS, Malik RK, 2002. Isoproturon resistant *Phalaris minor* and its response to alternate herbicides. Weed Technol 16: 116-123. https://doi.org/10.1614/0890-037X(2002)016[0116:IRLCPM]2.0.CO;2

Chhokar RS, Sharma RK, Chauhan DS, Mongia AD, 2006. Evaluation of herbicides against *Phalaris minor* in wheat in northwestern Indian plains. Weed Res 46: 40-49. https://doi.org/10.1111/j.1365-3180.2006.00485.x

Chhokar RS, Singh S, Sharma RK, 2008. Herbicides for control of isoproturon-resistant littleseed canarygrass (*Phalaris minor*) in wheat. Crop Prot 27: 719-726. https://doi.org/10.1016/j.cropro.2007.10.004

Crooks HL, York AC, Jordan DL, 2003. Wheat tolerance and Italian ryegrass control by AE F130060 00 plus AE F115008 08 mixed with other herbicides. Weed Technol 17: 881-889. https://doi.org/10.1614/wt-03-030R

Crooks HL, York AC, Jordan DL, 2004. Wheat tolerance to AE F130060 00 plus AE F115008 00 as affected by time of application and rate of the safener AE F107892. Weed Technol 18: 841-845. https://doi.org/10.1614/WT-03-122R1

Das TK, 2014. Herbicide selectivity in plants-Weed science: Basics and applications, 1st Edn. Jain Brothers, New Delhi, 438 pp.

Dhawan RS, Bhaskar P, Chawla S, 2010. Effect of pinoxaden on the seedling growth and chlorophyll development of the fenoxaprop-p-ethyl susceptible and resistant biotypes of *P. minor* and wheat. Indian J Weed Sci 42: 52-55.

Grey TL, Bridges DC, 2003. Alternatives to diclofop for the control of Italian ryegrass (*Lolium multiflorum*) in winter wheat (*Triticum aestivum*). Weed Technol 17:219-223. https://doi.org/10.1614/0890-037X(2003)017[0219:ATDFTC]2.0.CO;2

Grey TL, Cutts GS, Sosnoskie L, Culpepper AS, 2012. Italian ryegrass (*Lolium perenne*) control and winter wheat response to POST herbicides. Weed Technol 26: 644-648. https://doi.org/10.1614/WT-D-12-00046.1

Hofer U, Muehlebach M, Hole S, Zoschke A, 2006. Pinoxaden- for broad spectrum grass weed management in cereal crops. J Plant Dis Prot 20: 989-995.

Kaur N, Kaur T, Kaur S, Bhullar MS, 2016. Development of cross resistance in *Phalaris minor* Retz. in Punjab. Agric Res J 53: 69-72. https://doi.org/10.5958/2395-146X.2016.00011.9

Kieloch R, Domaradzaki K, Gornial J, 2006. Pinoxaden -a new active ingredient for grass weed control in cereals of South-West Poland. J Plant Dis Prot 20: 1067-1072.

Kleeman SGL, Gill GS, 2008. Applications of metribuzin for the control of rigid brome (*Bromus rigidus*) in no-till barley crops of Southern Australia. Weed Technol 22: 34-37. https://doi.org/10.1614/WT-07-017.1

Lemerle D, Hinkley RB, Kidd CR, Leys AR, 1986. Symptoms of injury caused by herbicides in wheat and barley. Advisory Bull (Dept. Agriculture, New South Wales) 4: 1-14.

Levy SJ, 2008. Environmental Protection Agency's records disposition schedule PEST 361 scientific data reviews HED (health effects division) records center - File R158148, 5 pp.

Runyan TJ, Mcneil WK, Peeper TF, 1982. Differential tolerance of wheat cultivars to metribuzin. Weed Sci 30: 94-97.

SAS, 2001. SAS User's Guide. SAS Institute, Cary, NC (USA).

Schroeder J, Banks PA, Nichols RL, 1986. Soft red winter wheat (*Triticum aestivum*) cultivar response to metribuzin. Weed Sci 34: 66-69.

Senseman SA, 2007. Herbicide handbook, 9th Edn. Am Weed Sci Soc, Champaign, IL, USA, 458 pp.

Shaner DL, 2014. Sulfosulfuron. In: Herbicide handbook, 10th Edn. Am Weed Sci Soc, Champaign, IL, USA. pp: 427-428.

Shaw DR, Wesley MT, 1991. Wheat (*Triticum aestivum*) cultivar tolerance and Italian ryegrass (*Lolium multiflorum*) control with diclofop, BAY SMY 1500, and metribuzin. Weed Technol 5: 776-781.

Singh G, Singh VP, Singh M, Singh RK, 2003. Effect of doses and stages of application of sulfosulfuron on weeds and wheat yield. Indian J Weed Sci 35: 183-185.

Singh S, Kirkwood RC, Marshall G, 1999. Biology and control of *Phalaris minor* Retz. (littleseed canarygrass) in wheat. Crop Prot 18: 1-16. https://doi.org/10.1016/S0261-2194(98)00090-8

Singh S, Singh S, Sharma SD, Punia SS, Singh H, 2005. Performance of tank mixture of metribuzin with clodinafop and fenoxaprop for the control of mixed weed flora in wheat. Indian J Weed Sci 37: 9-12.

Singh S, Singh K, Punia SS, Yadav A, Dhawan RS, 2011. Effect of stage of *Phalaris minor* on the efficacy of Accord plus (fenoxaprop+metsulfuron, readymix). Indian J Weed Sci 43: 23-31.

Tickes B, 2000. Evaluation of herbicides for the control of littleseed canarygrass in wheat. Forage and grain report. University of Arizona, College of Agriculture and Life Sciences.

Tottman DR, 1980. Varietal differences in the tolerance of cereals to herbicides. Winter Wheat, Crop Conference, p. 68.

Yuan X, Guo P, Qi X, Ning N, Wang H, Wang X, Yang Y, 2013. Safety of herbicide Sigma Broad on *Radix isatidis* (*Isatis indigotica* Fort.) seedlings and their photosynthetic physiological responses. Pestic Biochem Physiol 106: 45-50. https://doi.org/10.1016/j.pestbp.2013.04.002

Zadoks JC, Chang TT, Konzak CF, 1974. A decimal code for the growth stages of cereals. Weed Res 14: 415-421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x